Fluorescence is a popular family of techniques used to study membranes, however recent systematic studies show that fluorescent probe behaviour can be altered by membrane composition, probe concentration, and the presence of other probes. Using deuterium nuclear magnetic resonance spectroscopy (^{2}H NMR), we found that trace amounts of the carbocyanine probe DiIC12 are enough to alter phase coexistence behaviour of 30:30:35 DPPC-D62:DOPC:cholesterol membranes, while other probes like Laurdan, Naphthopyrene, and another carbocyanine probe DiOC18, did not affect the membrane appreciably. Laurdan is particularly well suited to the study of phase separation in lipid membranes. It partitions equally well into ordered and disordered lipid phases, and displays a phase-dependent emission spectral shift. Laurdan general polarization (GP) parameter, which characterizes said emission spectral shift, has been used to characterize membrane fluidity. We examine the relationship between Laurdan GP and ^{2}H NMR order parameters.

2572-Pos Board B264

Chemical Stress and the Cell Envelope: The Phospholipid Fraction

Samuel Furse, Anton I. de Kroon, J. Antoinette Killian.
Scheikunde, Universiteit Utrecht, Utrecht, Netherlands.

Evidence from physical studies of lipids in the last decade suggests more clearly than ever before, that the phospholipid fraction of cell membranes is not an inert participant in the cell, but a sensitive, dynamic system. The simplest living examples of this are prokaryotic micro-organisms such as *E. coli*. In this project, we explore the effect of growing E. coli in the presence of chemical stress (*e.g.n*-butanol) using updated lipidomics techniques (TLC, MS and NMR) and several biophysical techniques (broad-line 31P NMR and differential scanning calorimetry). *n*-Butanol is an important product of industrial micro-organismal growth, with economically widespread uses, such as an alternative to petrol.

The combination of different experimental approaches in a chemical-biology-type strategy is designed to deliver a more fundamental understanding concerning the physical role of lipids in prokaryotes with state-of-the art accuracy. This in turn allows us to generate understanding about the effect of chemical stress agents, such as *n*-butanol, on the cell envelope.

2573-Pos Board B265

Profiling the Dielectric Constant at the Membrane-Peptide Interface using Ionizable EPR Probes

Matthew Donohue, Maxim A. Voynov, Alex I. Smirnov,
Tatyana I. Smirnova.
Chemistry, North Carolina State University, Raleigh, NC, USA.

Polarity, electric potentials, and hydration are the major physico-chemical characteristics of lipid membranes that govern membrane-protein and protein-protein interactions as well as small molecules transport. Insertion of transmembrane proteins perturbs membrane structure altering local dielectric environment and hydration at the membrane-protein interface. The significance of distorting local membrane structure at the lipid-protein interface for modulating protein-protein interactions should not be overlooked. In this work we report on employing pH-sensitive ionizable EPR labels to profile a heterogeneous dielectric environment along the α-helix of a WALP peptide integrated in a lipid bilayer. Labels were attached to two cysteine residues positioned equidistant from the center of the peptide so that the primary sequence of each peptide is palindromic, thus insuring symmetric location of the labels with respect to the bilayer center. The change in protonation state of the nitroxide was directly observed by EPR. Q-band double electron-electron resonance (DEER) experiments were carried out to determine the distance between spin labels when imbedded in lipid bilayers to provide information about the label location. Thus, for the first time measurements of local electrostatics at peptide-bilayer interface were based on direct distance measurements rather than on assumptions on the probe location. Two pH sensitive spin labels, methanethiosulfonic acid S-(1-oxyl-2,2,3,5,5-pentamethyl-imidazolidin-4-ylmethyl) ester (IMTSL) and S-4-(4-(dimethylamino)-2-ethyl-5,5-dimethyl-1-oxyl-2,5-dihydro-1H-imidazol-2-yl) benzylmethanethiosulfonate (IKMTSL), with intrinsic pKa's differing by approximately 2 pH units were used to expand the pH range of the titration experiments. This provided the opportunity to vary the lipid composition in order to investigate effect of the surface charge on dielectric profile at peptide-membrane interface. Water penetration at the peptide-membrane interface was assessed by hyperfine sublevel correlation spectroscopy (HYSCORE) experiment in which the hyperfine coupling between the nitroxide and hydrogen/deuterium atom of water is measured. Supported by NSF-0843632 to TIS.

2574-Pos Board B266

Screening the Dynamics of Membrane Constituents in Intact Microalgal Cells by Solid-State NMR

Alexandre A. Arnold[1], Bertrand Genard[1], Andre L.D.A.G. Da Silva[1], Réjean Tremblay[2], Dror E. Warschawski[3], Francesca Zito[3], **Isabelle Marcotte**[1].
[1]Department of Chemistry, Université du Québec à Montréal, Montréal, QC, Canada, [2]Institut des Sciences de la Mer, Université du Québec à Rimouski, Rimouski, QC, Canada, [3]Institut de Biologie Physico-Chimique, CNRS-Université Paris Diderot, Paris, France.

Microalgae are unicellular organisms protected by a lipid-rich plasma membrane. In most species, this bilayer is covered by a cellulose, carbonate or silica wall. Microalgae are at the basis of the aquatic food chain; therefore the action of contaminants from human activities can impact all the trophic food web. To exert their biological effect, these molecules can target or traverse the microalgal membranes to access internal activity sites, with important consequences on cell survival. Solid-state nuclear magnetic resonance (SS-NMR) is a non- intrusive method that can provide information at the molecular level on the membrane constituents. Because of the complex composition of natural membranes, SS-NMR generally relies on the use of mimetics made of phospholipids. The objective of our work was thus to provide a tool to probe all membrane components by ^{13}C SS-NMR using isotopically-labeled intact microalgae. To do so, we have established a strategy to sort specific constituents according to their dynamics. Using experiments based on through-space (cross polarization) or through-bond (RINEPT) magnetization transfer, rigid and dynamic molecules, respectively, can be identified and studied. For this purpose, we have developed protocols to ^{13}C-enrich microalgae up to an average labeling of 90%. Our ^{13}C SS-NMR study of saltwater (*I. galbana*, *C. gracilis*) and freshwater (*C. vulgaris*, *C. reinhardtii*) microalgae revealed important differences in the dynamics of their cell wall constituents. Notably, *C. vulgaris* used in biofuel production presents the most dynamic membrane due to its high lipid content. Also, the integrity of species without cell walls, such as *I. galbana*, seems to be ensured by a more rigid organization. Our dynamic filter strategy was also tested to verify the effect of nanocrystalline cellulose and could reveal membrane stiffening at high nanoparticle concentrations.

2575-Pos Board B267

Docosahexaenoic Acid Affects Gel Phase by Increasing Tilt Angle

Chai Lor, Linda S. Hirst.
School of Engineering, University of California Merced, Merced, CA, USA.

The physical properties of docosahexasenoic acid (DHA) have dramatic effects on lipid bilayer phase behavior which could be a link to beneficial health. Its highly unsaturated structure makes fatty acid highly flexible and with a low transition temperature (Tm) of −80°C. In this investigation, we explore a binary system composed of the saturated lipid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1-dipalmitoyl-2-docohaxeanoyl-sn-glycero-3-phosphoethanolamine (DHA-PE, 22:6) using small angle x-ray scattering (SAXS) and atomic force microscopy (AFM). Solution SAXS is a powerful tool for structural characterization and was used to identify the different phases (Lβ' and Lα) co-existing within the membrane. In a complementary study, AFM was used to investigate the morphology of coexisting domains. In a surprising result, we observed that the DHA-PE lipid used in this study induced phase separation at concentrations as low as 0.25mol% into a DHA-PE rich liquid crystalline (Lα) phase and a DHA-PE poor gel (Lβ') phase. Also unexpectedly the thickness of the gel phase is thinner than the fluid phase as shown in the d-spacing and electron density profile. We anticipated that DHA-PE would remove or decrease the angle of tilted Lβ' phase that is composed of high concentration of DPPC, but instead it does the opposite and increase the tilt angle. However, DHA-PE still maintain its thermal property and does lower the Tm of the Lβ' phase.

2576-Pos Board B268

Lipopolysaccharide Induced Dynamic Lipid Organizations: Lipid Tubules, Membrane Perforations and Multi-Lamellar Stacking

Peter G. Adams[1], Kirstie Swingle[1,2], Loreen Lamoureux[3], Harshini Mukundan[2,4], Gabriel A. Montaño[1].
[1]Center for Integrated Nanotechnologies, Los Alamos National Laboratory, Los Alamos, NM, USA, [2]New Mexico Consortium, Los Alamos, NM, USA, [3]Center for Biomedical Engineering, University of New Mexico, Albuquerque, NM, USA, [4]Physical Chemistry and Applied Spectroscopy, Los Alamos National Laboratory, Los Alamos, NM, USA.

Supported lipid bilayer assemblies (sLBAs) are generally thought of as relatively stable, predictable model membranes, relevant to a biological system.

Lipopolysaccahride (LPS) is a unique lipoglycan, with two major functions: (i) as a major component of the outer membrane of Gram-negative bacteria and (ii) as a highly potent human toxin when released from cells into solution ("endotoxin"). Divalent cations have long been known to neutralize and stabilize LPS in the outer membrane, whereas LPS in the presence of monovalent cations forms highly mobile negatively-charged aggregates. We report fluorescence microscopy and atomic force microscopy analysis of the interaction between soluble LPS and a single component fluid-phase sLBA. Three remarkably different deformations are induced by LPS on the simple lipid membrane, dependent on cation availability. LPS is an amphiphile that spontaneously inserts into the outer leaflet of lipid bilayers to bury its hydrophobic lipidic domain and expose the hydrophilic polysaccharide chain to the exterior polar solvent. Net negative (LPS-Na^+) induces membrane curvature due to electrostatic repulsive effects between clustered LPS. This leads to (1) the growth of 100μm-long flexible lipid tubules from surface associated lipid vesicles and (2) destabilization of the sLBA leading to micron-sized hole formation. In contrast, Ca^{2+} promotes self-association and bridging of LPS, and (LPS-Ca^{2+}) induces (3) growth of 100μm-wide planar single- or multi-lamellar sheets of lipid and LPS from surface associated lipid vesicles that exhibit 2-D membrane fluidity and represent a potential means of organizing layer-by-layer membrane construction. Our findings have important implications about the physical interaction of LPS and lipids and the potential of using LPS and other amphiphilic materials as membrane soft-lithography tools.

2577-Pos Board B269

Lipid Tilt Regulates Ripple Phase Behavior in Lipid Bilayer

Padmini Rangamani, Shachi Katira, Berend Smit, George Oster.
UC Berkeley, Berkeley, CA, USA.

Continuum modeling of lipid bilayers provides insight into the physics underlying geometric changes to the shape of the membrane in response to biological processes. The Helfrich model has been the gold standard for many years and applies only to length scales larger than that of the thickness of the bilayer. For small length scale processes, orientation of the lipid, characterized by 'lipid tilt', is a suitable fundamental degree of freedom. In this work, we develop a continuum model with lipid tilt as the key degree of freedom. Using local force balance, we derive the equations of motion associated with the membrane. We use this model to study the characteristics of ripple phases in bilayers. Comparing the continuum model to coarse-grained simulations, we find that the tilt degree of freedom is important to allow for ripple formation in bilayer membranes.

2578-Pos Board B270

Macroscopic Phase Separation, Modulated Phases, and Microemulsions: a Unified Picture of Rafts

Roie Shlomovitz[1], Lutz Maibaum[2], Michael Schick[1].
[1]Physics, University of Washington, Seattle, WA, USA, [2]Chemistry, University of Washington, Seattle, WA, USA.

We are motivated by the observation that coupling of the height and the composition of fluctuating membranes can reduce the line tension between regions of different components. Consequently, instead of undergoing a transition from a disordered fluid to liquid-ordered and liquid-disordered phases, the system undergoes a transition to a modulated phase. The disordered fluid is also affected, displaying the behavior of a microemulsion, one suggested as a model for rafts in the plasma membrane. We consider a model of a multi-component symmetric bilayer which highlights the competition between the tendency to phase separate as the temperature is reduced, and to form modulated phases as the line tension is decreased. We simulate the model on a finite-size membrane and obtain its phase diagram. At low temperature, the system undergoes macroscopic phase separation. As temperature increases, the system can evolve in two ways. If the line tension is sufficiently large, the system undergoes a transition to an ordinary disordered fluid. However if sufficiently small, the system undergoes a first-order transition to a modulated phase. Only upon a further increase of temperature does the system undergo a transition to a fluid phase, and this fluid is a microemulsion. In this disordered phase, the fluctuating regions rich in one component or the other are clearly seen in the simulation. The model provides a unified picture of the relationship between observations, in vitro and in vivo, of macroscopic phase separation and of modulated phases in bilayers and their relation to rafts. It lends support to the suggestion that rafts can be identified with a microemulsion whose characteristic length, the square root of the ratio of bending modulus to surface tension, is on the order of 100 nm in the plasma membrane.

2579-Pos Board B271

The Structural Basis of Cholesterol Accessibility in Membranes

Brett N. Olsen[1], Agata A. Bielska[1], Tiffany Lee[1], Michael D. Daily[2], Douglas F. Covey[3], Paul H. Schlesinger[4], Nathan A. Baker[2], Daniel S. Ory[1].
[1]Medicine, Washington University, St. Louis, MO, USA, [2]Knowledge Discovery and Informatics, Pacific Northwest National Laboratory, Richland, WA, USA, [3]Developmental Biology, Washington University, St. Louis, MO, USA, [4]Cell Biology, Washington University, St. Louis, MO, USA.

While the majority of free cellular cholesterol is present in the plasma membrane, cholesterol homeostasis is principally regulated through sterol-sensing proteins that reside in the cholesterol-poor endoplasmic reticulum. In response to acute cholesterol loading or depletion, there is rapid equilibration between the ER and plasma membrane cholesterol pools, suggesting a biophysical model in which the availability of plasma membrane cholesterol for trafficking to internal membranes modulates ER membrane behavior. Previous studies have predominantly examined cholesterol availability in terms of binding to extra-membrane acceptors, but have provided limited insight into the structural changes underlying cholesterol activation. In the present study we use both molecular dynamics simulations and experimental membrane systems to examine the behavior of cholesterol in membrane bilayers. We find that cholesterol depth within the bilayer provides a reasonable structural metric for cholesterol availability and that this is correlated with cholesterol-acceptor binding. Further, the distribution of cholesterol availability in our simulations is continuous rather than divided into distinct available and unavailable pools. This data provides support for a revised cholesterol activation model in which activation is driven not by saturation of membrane-cholesterol interactions but rather by bulk membrane remodeling that reduces membrane-cholesterol affinity.

2580-Pos Board B272

Keeping Order While Moving Fast: Ergosterol Pairs Lead to Dynamic Networks in Lipid Membranes

Juan M. Vanegas[1], **Roland Faller**[2], Marjorie L. Longo[2].
[1]Sandia National Lab, Albuquerque, NM, USA, [2]UC Davis, Davis, CA, USA.

We analyze the dynamic structure in lipid-ergosterol membranes by means of time-dependent pair-correlation functions obtained from molecular dynamics simulations. We observe that ergosterol molecules form transient pairs with lifetimes in the nanosecond-microsecond range. These sterol pairs are sufficiently long-lived to form linear sterol clusters (> 4 molecules) as the ergosterol concentration increases, and at high enough concentrations (> 30 mol %) these linear clusters turn into larger networks. Because of the high mobility of the sterols, as well as the dynamic nature of their pair interaction, these sterol networks are constantly reshaping.

2581-Pos Board B273

Multi-Color, Live Super-Resolution Microscopy Reveals the Timescale and Potential of Mean Force for Co-Clustering Between the B cell Receptor and Lyn Kinase

Matthew B. Stone, Sarah L. Veatch.
Biophysics, University of Michigan, Ann Arbor, MI, USA.

The super-resolution techniques STORM and PALM allow the nanometer scale localization of fluorescent probes in both live and fixed cells. In fixed cells, robust quantification of interactions between proteins is accomplished by cross correlating the reconstructed images of spectrally separated probes. Here, we extend this technique to live cells using simultaneous STORM and PALM measurements quantified using time resolved cross correlation with 20μs temporal resolution. We demonstrate that the potential of mean force between two labeled proteins can be determined even when objects have significant diffusion. We apply this technique to the activation of B cell receptor (BCR) and investigate the timescale and magnitude of co-clustering between the BCR and the Src kinase Lyn. We simultaneously compare quantitative measurements of receptor clustering, receptor-Lyn co-clustering, and protein mobility. We find that Lyn is recruited to BCR clusters with a potential of mean force greater than 1kT after stimulation with multivalent antigen, concurrent with BCR self-clustering and slowdown. We correlate two distinct lipid modified PALM probes with the B cell receptor in live cells. A saturated lipid moiety is recruited to BCR clusters with a potential of mean force of greater than 0.5kT, while a branched and unsaturated lipid moiety is not recruited to BCR clusters. These observations confirm that BCR clustering leads to the formation of a stable lipid domain enriched in saturated components, in quantitative agreement with our observations in chemically fixed cells. In ongoing work, we are using these experimental and analytical methods to quantify how lipids mediate interactions between proteins involved in early stages of BCR cell signaling.